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Nucleic acids extraction from single cell using MasterPure Complete DNA purification (Epicenter) V.2

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We use this protocol and it's working

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Abstract

Radiolaria are protists which can't be cultivated. These microoganisms have to be isolated by single-cell for genetic identification and it can be difficult to get clean DNA.
Here we optimized a DNA extraction protocol from protist single-cell.
It works very well on single-cell Radiolaria, Foraminifers, but also Ciliates, Dinoflagelletes, Diatoms.

Materials

Material and Equipment :

- Glass bent micropipettes.
- Silicone Tubing (Cole-Parmer, Tube silicone platinium LS T17 ref. **FV-96410-17**
- StereoMicroscope or binocular microscope.
- Petridishes Diameter 70mm.
- Centrifuge 5417R (Eppendorf)
- Thermomixer Eppendorf

Kit, Reagents and Chemicals :

- **MasterPure Complete DNA and RNA purification kit** (Epicenter Illumina, ref MC85200)
  Enzymes (Proteinase K, RNase, DNase) are stored at -20°C
  Other reagents from the kit are stored at room temperature.
- Ethanol absolute, molecular biogy quality.
- Propan-2-ol quality molecular biology.
- 0,22µm filtered Seawater or artificial Seawater.
- Ice for enzyme storage during labwork.

Safety warnings

Wear labcoat, gloves.
Decontaminate all the surface area, and equipments (rotor, racks, pipettes...) with Ethanol 70% and DNA away.
Works with filter tips.

Before start

Prepare Ethanol 70% from absolute Ethanol : Mix 35 mL of absolute Ethanol with 15 mL nuclease-free water in a Falcon 50mL Store at -20°C.

1. Cell Isolation

1 Isolate individually protist cells (at least 50µm in length) using a glass bent micropipette under a binocular microscope.

2 Wash each cell in three successive baths of 0.22µm-filtered and sterile seawater.

3 Transfer subsequently cells in a 1.5mL sterile microtube.

4 Add 30 µL of lysis buffer (Tissue and Cell Lysis Solution from MasterPureTM DNA and RNA Purification Kit, Epicenter) and store at -20°C.

2. Cell lysis

5 Pellet cells by centrifugation (2 min at Vmax), throw the supernatant, let ~25-30 µL of liquid.

6 Dilute 1 µL of Proteinase K in 300 µL de lysis solution Tissue et Cellule for each sample. Vortex 10 sec for resuspending cells (facultative).

7 Add 300 µL of mix Proteinase K + lysis solution Tissue et Cellule in each sample. Vortex.

8 Incubate 15 min at 65°C , 1000 rpm. Put samples in ice 3-5 min.

3. Total nucleic acids precipitation

9 Add 150 µL MPC reagent to 300 µL of lysed sample. Vortex.

4-+ 500 µL Propan-2-ol. Mix per inversion.
5-
6
7-+ 500 µL d'EtOH 70%. Don't vortex, mix gently the support. Spin 5min at Vmax ;at 12°C.
8-Discard a maximum of supernatant with precaution, without touching the pellet.

9-Let dry 5-10 min at RT.
10-

10 Spin 10 min at 11 000 g, 12°C. If there is no pellet, add more 25 µL MPC buffer and spin again 10 min at 11 000 g, 12°C.

11 Transfer the supernatant ion a new clean microtube (1,5 mL), discard the pellet.
(To keep the squeletton, keep the tube with the pellet, add 500 µL MilliQ Water and store at -20°C).

12 Add 500 µL of Isopropanol. Mix per inversion. Spin 10min at Vmax, 12°C.

13 Discard the supernatant with precaution, without touching the pellet.

14 Add 500 µL of Ethanol 70%. Don't vortex, mix gently the support. Spin 5min at Vmax ;at 12°C.

15 Discard a maximum of supernatant with precaution, without touching the pellet.

16 Let dry 5-10 min at room temperature..

17 Elute in 25 µL of TE1x buffer.
Vortex and spin shortly.
Store at - 80°C.